

University of Groningen

An Acrasin-Like Attractant from Yeast Extract Specific for Dictyostelium lacteum

Mato, José M.; Haastert, Peter J.M. van; Krens, Frans A.; Konijn, Theo M.

Published in:
Developmental Biology

DOI:
[10.1016/0012-1606\(77\)90229-9](https://doi.org/10.1016/0012-1606(77)90229-9)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1977

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Mato, J. M., Haastert, P. J. M. V., Krens, F. A., & Konijn, T. M. (1977). An Acrasin-Like Attractant from Yeast Extract Specific for Dictyostelium lacteum. *Developmental Biology*, 57(2).
[https://doi.org/10.1016/0012-1606\(77\)90229-9](https://doi.org/10.1016/0012-1606(77)90229-9)

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

An Acrasin-Like Attractant from Yeast Extract Specific for *Dictyostelium lacteum*

JOSÉ M. MATO, PETER J. M. VAN HAASTERT,
FRANS A. KRENS, AND THEO M. KONIJN

Cell Biology and Morphogenesis Unit, Zoological Laboratory, University of Leiden, Kaiserstraat 63, Leiden, the Netherlands

Received October 18, 1976; accepted in revised form December 15, 1976

The transition of the unicellular to the multicellular stage in *Dictyostelium lacteum* is not mediated by cyclic AMP. The attractant for aggregative amoebae of this cellular slime mold species was isolated from yeast extract and purified more than 1000-fold without a significant loss of activity. Several characteristics of the chemotactic molecule specific for *D. lacteum* are reported, and the presence of an inactivating enzyme has been demonstrated.

INTRODUCTION

Chemotaxis mediates cell aggregation in the cellular slime molds (1). In 1967, the chemotactic molecule for aggregative amoebae of *D. discoideum* was purified from a bacterial extract and identified as cyclic AMP (2) a compound which was shown to be the acrasin of this species (3, 4). *D. rosareum*, *D. purpureum* and *D. mucoroides* are also attracted by this cyclic nucleotide (5); acrasins of all other species wait for identification. The attractant folic acid is not species-specific and is generally more active in the vegetative stage than in the aggregative stage (6); its role, therefore, is probably more closely related to food searching than to cell aggregation. Recently, the acrasin of *Polysphondylium violaceum* has been purified, and, although its final identification has not been achieved yet, it probably is a small peptide (7). To understand the molecular mechanism involved during cell aggregation, it is necessary to identify the different acrasin molecules; therefore, purification and preliminary characterization of an acrasin-like substance for *D. lacteum* derived from yeast extract was carried out.

RESULTS AND DISCUSSION

Amoebae of *D. lacteum* were grown on agar in association with *Escherichia coli*, harvested, freed of bacteria by centrifugation, and, after being resuspended in a 1% salt solution (5×10^6 cells/ml), were placed in small drops on a hydrophobic agar surface (8). The small amoebal populations were incubated at 22°C in darkness, and the different yeast extract fractions were tested for their chemotactic activity when the amoebae were close to aggregation. Drops, 0.1 μ l, of the testing material were placed near (100–300 μ m) the sensitive amoebae twice, at 5-min intervals. The amoebal response was monitored 30 min after the last deposition and was called positive when at least twice as many cells were pressed against the edge closest to the attracting drop than were pressed against the opposite edge (8).

Because of the low yield of attractant isolated from amoebae, yeast extract which gave a strong chemotactic response was used as source of the active component. Yeast extract (Difco) in water (600 mg/ml) was precipitated with 95% ethanol (45% final concentration) and centrifuged at 2300g for 10 min; the supernatant was

filtered (0.45- μ m Millipore filter) and, after being dried at 50°C, was dissolved in 10 mM phosphate buffer, pH 6.0 (500 mg/ml). Twenty milliliters of this solution (fraction I) which strongly attracted amoebae of *D. lacteum* was mixed with DEAE-Sephadex A-25 (1.0 g of Sephadex/ml) equilibrated with 10 mM phosphate buffer, pH 6.0, and was vigorously shaken for 30 min at 22°C. The chemotactic activity was not bound and could be recovered by washing the gel three times with 10 ml of the same buffer on a Buchner funnel (fraction II). Some minor chemotactic activity could be removed from the gel by washing it with 0.3 M NaCl; this negatively charged fraction had a molecular size larger than 2200 daltons and was not further purified. Fraction II was treated with SP-Sephadex C-25 (0.4 g of Sephadex/ml) equilibrated in 10 mM phosphate buffer, pH 6.0, shaken 30 min, and filtered as before. The eluate (fraction III), which contained all the chemotactic activity, was freeze-dried and dissolved in 10 ml of water, and 3-ml samples were chromatographed on Bio-Gel P2 (Fig. 1a). Tubes 16–20 contained nearly all the chemotactic activity and were pooled, freeze-dried, and dissolved in 2 ml of water (fraction IV). A minor peak of activity, although not always detected, was eluted with the void volume (molecular size larger than 2200 daltons) and was not purified further. Samples, 1.5 ml, from fraction IV were chromatographed on Sephadex G-10 (Fig. 1b), and the chemotactic activity was recovered as a single peak in tubes 15 and 16 which were freeze-dried (fraction V). About 7.5 mg of dry purified substance was obtained from 14 g of original yeast extract. This substance gave at a concentration of 0.5 mg/ml, about the same chemotactic activity as fraction I (500 mg/ml), indicating a purification of at least 1000-fold. At a higher concentration (1.0 mg/ml), the chemotactic response was not only in the direction of the attracting drop but was also centrifugal, as occurs in small

populations of *D. discoideum* amoebae near concentrated cyclic AMP drops (8). The threshold concentration for chemotaxis was 0.01 mg/ml (Fig. 2). All following experiments were carried out with this purified active substance.

The attractant was not bound to SP-Sephadex C-25 equilibrated in phosphate buffer, pH 5.0, or to DEAE-Sephadex A-25 equilibrated in phosphate buffer, pH 6.0. Binding to DEAE-Sephadex A-25 equilibrated in phosphate buffer occurred at pH 7.5, which indicates a negative charge of the attractant. With polyacrylamide electrophoresis [7% acrylamide, separating gel pH 8.9, buffer system pH 8.3 (9)] the attractant moved at the same speed as the tracking dye (bromphenol blue), which means that the compound is negatively charged. The spectral analysis of the active substance in 10 mM phosphate buffer, pH 6.0, showed two peaks of maximal ab-

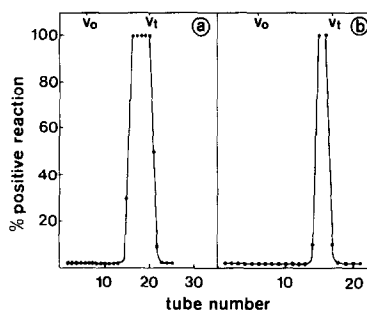


FIG. 1. (a) Chromatogram of fraction III from yeast extract through a Bio-Gel P-2 column equilibrated with water (bed volume 180 ml; 6.7 ml/hr; 9 ml/tube). The separation range of this gel is 2200–200 daltons, and the chemotactic activity eluted just ahead of the total bed volume (v_0); the estimated size for this attractant is about 200 daltons. (b) Chromatogram of fraction IV through a Sephadex G-10 column (bed volume 41 ml; 11 ml/hr; 2.4 ml/tube). The chemotactic activity was eluted just ahead of v_1 , which again indicates a molecular size in a range similar to that in Fig. 1a. By rechromatography of fraction V in Sephadex G-10 equilibrated in 10 mM phosphate buffer, pH 6.0, it was shown that the active substance was not retarded when chromatographed in water. The chemotactic activity is expressed as the percentage of amoebae drops that react positively. V_0 , void volume.

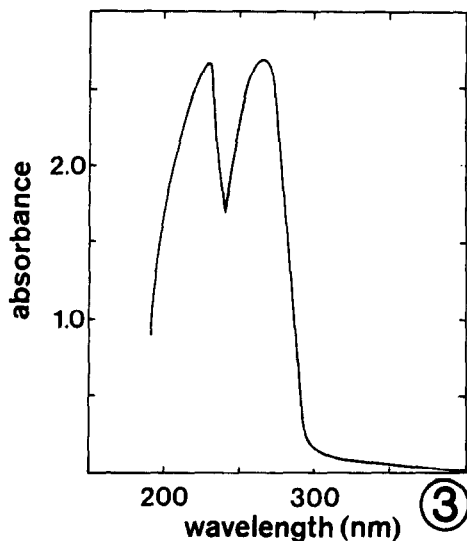
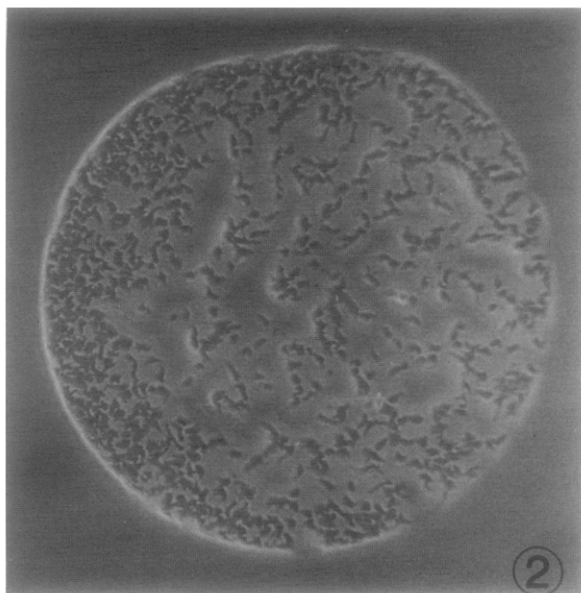


FIG. 2. Chemotactic response of *D. lacteum* amoebae to the threshold concentration of the purified attractant. The testing material was placed to the left of the responding drop (see text). Magnification: 80 \times .

FIG. 3. Ultraviolet scan of fraction V in 10 mM phosphate buffer, pH 6.0, 0.25 mg/ml.

sorbance at wavelengths of, respectively, 230 and 265 nm (Fig. 3). It was positive on the test of Lowry *et al.* (10), and 0.38 mg of active substance gave a reaction comparable to that of 0.54 mg of bovine serum albumin. When 100 μ l of the active substance (1.0 mg/ml) in 10 mM phosphate buffer, pH 6.0, was incubated for 90 min at 37°C with Pronase (Merck, 0.2 mg or 2.0 mg/ml final concentration) and the reaction was stopped by boiling for 5 min, no loss of activity was observed; this indicates that the attractant probably is not a Pronase-sensitive peptide. Also, after incubation of the active substance with beef heart cyclic nucleotide phosphodiesterase (Boehringer, 0.2 mg/ml final concentration) for 90 min under similar conditions, no loss of activity was observed.

To obtain an inactivating enzyme (acrasinase) of the attractant, 10 ml of an amoebal suspension of *D. lacteum* (10^7 cells/ml) was shaken in a 1% salt solution for 1 hr at 22°C; after centrifugation, the supernatant was freeze-dried, dissolved in 1 ml of 10 mM phosphate buffer, pH 6.0, and stored

at -20°C . Samples of it, 20 μ l, were added to 100 μ l of the active substance (1.0 mg/ml) in 10 mM phosphate buffer, pH 6.0; a complete inactivation was obtained within a 30-min incubation at 37°C. When samples of the freeze-dried and dissolved amoebal supernatant were boiled for 5 min and added to the chemotactic substance, the chemotactic activity remained constant, even after a 90-min incubation at 37°C. These results suggest the presence of an acrasinase secreted by the amoebae of *D. lacteum* to the medium. Acrasinase activity was also found in amoebal supernatants from *D. discoideum*, *D. minutum*, and *P. violaceum*. However, the attractant for *D. lacteum* did not show any chemotactic activity with *D. discoideum*, *D. minutum* and *P. violaceum* in either vegetative or preaggregative stage. Amoebae of *D. lacteum* reacted chemotactically to the attractant only in the preaggregative stage, but not during the vegetative stage, even at 1.0 mg/ml.

In conclusion, the following characteristics suggest that the chemotactic sub-

stance purified from yeast extract is an obvious candidate for the acrasin of *D. lacteum*: small molecular size (Fig. 1), water-diffusible, heat stable, negatively charged, probably aromatic, different from all other known attractants, specific for *D. lacteum*, active at physiological concentrations only during the preaggregative stage, and inactivated by an enzyme secreted to the medium by the amoebae.

We thank Elsa Boon for technical assistance.

REFERENCES

1. BONNER, J. T. (1947). *J. Exp. Zool.* **106**, 1-26.
2. KONIJN, T. M., VAN DE MEENE, J. G. C., BONNER, J. T., and BARKLEY, D. S. (1967). *Proc. Nat. Acad. Sci. USA* **58**, 1152-1154.
3. BARKLEY, D. S. (1969). *Science* **165**, 1133-1134.
4. KONIJN, T. M., CHANG, Y. Y., and BONNER, J. T. (1969). *Nature (London)* **224**, 1211-1212.
5. KONIJN, T. M. (1972). In "Advances in Cyclic Nucleotide Research" (P. Greengard, and G. A. Robison, eds.), Vol. 1, pp. 17-31. Raven Press, New York.
6. PAN, P., HALL, E. M., and BONNER, J. T. (1975). *J. Bacteriol.* **122**, 185-191.
7. WURSTER, B., PAN, P., TYAN, G. G., and BONNER, J. T. (1976). *Proc. Nat. Acad. Sci. USA* **73**, 795-799.
8. KONIJN, T. M. (1970). *Experientia* **26**, 367-369.
9. GABRIEL, O. (1971). In "Methods in Enzymology" (W. B. Jakoby, ed.), Vol. 22, pp. 565-578. Academic Press, New York.
10. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J. (1951). *J. Biol. Chem.* **193**, 265-275.